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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 : A61K 49/02	A1	(11) International Publication Number: WO 88/ 01180 (43) International Publication Date: 25 February 1988 (25.02.88)

(21) International Application Number: PCT/US87/02076

(22) International Filing Date: 18 August 1987 (18.08.87)

(31) Priority Application Number: 897,455

(32) Priority Date: 18 August 1986 (18.08.86)

(33) Priority Country: US

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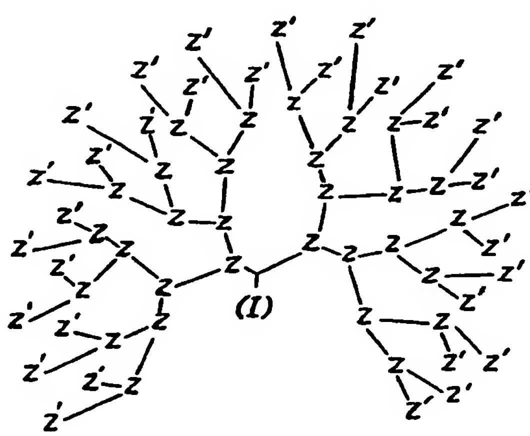
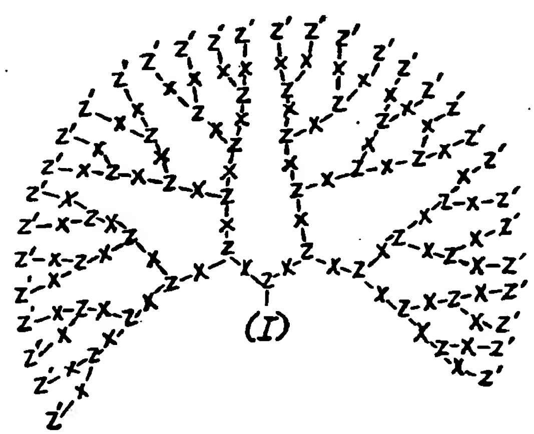
(81) Designated States: BR, JP.

Published

With international search report.
With amended claims.

(54) Title: STARBURST CONJUGATES





(57) Abstract

Starburst conjugates which are composed of at least one starburst polymer in association with at least one unit of a carried material have been prepared. These conjugates have particularly advantageous properties due to the unique characteristics of the starburst polymer.

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STARBURST CONJUGATES

The present invention concerns the use of dense star polymers as carriers for selected materials. In recent years polymers referred to as dense star polymers or starburst polymers have been developed. It has been found that the size, shape and properties of these dense star polymers or starburst can be molecularly tailored to meet specialized end uses. Starburst polymers have significant advantages which can provide a means for the delivery of high concentrations of carried material per unit of polymer, controlled delivery, targeted delivery and/or multiple species delivery or use.

In its broadest aspect, the present invention is directed to polymer conjugate materials comprising dense star polymers or starburst polymers associated with desired materials (hereinafter these polymer conjugates will frequently be referred to as "starburst conjugates" or "conjugates"), processes for preparing these conjugates, compositions containing the

conjugates, and methods of using the conjugates and compositions.

The conjugates of the present invention are suitable for use in a variety of applications where specific delivery is desired. In a preferred embodiment of the present invention, the starburst conjugates are comprised of one or more starburst polymers associated with one or more agents.

The starburst conjugates offer significant benefits over other carriers known in the art due to the advantageous properties of the starburst polymers. Starburst polymers exhibit molecular architecture characterized by regular dendritic branching with radial symmetry. These radially symmetrical molecules are referred to as possessing "starburst topology". These polymers are made in a manner which can provide concentric dendritic tiers around an initiator core. The starburst topology is achieved by the ordered assembly of uniform (within each tier) organic ~~repeating units in concentric, dendritic tiers around~~ an initiator core; this is accomplished by introducing multiplicity in a geometrically progressive fashion through a number of molecular generations. The resulting highly functionalized molecules generations have been termed "dendrimers" in deference to their branched (tree-like) structure as well as their oligomeric nature. Thus, the terms starburst oligomer and starburst dendrimer are encompassed within the term starburst polymer.

Covalent bridging of the starburst dendrimers through their reactive terminal groups produces a class of topological polymers, with size and shape controlled

domains, which are referred to as "starburst bridged dendrimers", which term is also encompassed within the term starburst polymer.

The following description of the figures aid in understanding the present invention.

Figure 1 depicts various generations of starburst dendrimers.

Figure 2A depicts a dendrimer having unsymmetrical (unequal) branch junctures.

Figure 2B depicts a dendrimer having symmetrical (equal) branch junctures.

The starburst polymers are illustrated by Figure 1 wherein I represents an initiator core (in this figure a tri-functional initiator core, shown by the far left drawing); Z represents a terminal group; shown in the first instance by the second drawing from the left, referred to as a starbranched oligomer; A, B, C, D, and E represent particular molecular generations of starburst oligomers, called dendrimers; and $(A)_n$, $(B)_n$, $(C)_n$, $(D)_n$, and $(E)_n$ represent starburst bridged dendrimers.

The starburst dendrimers are unimolecular assemblages that possess three distinguishing architectural features, namely, (a) an initiator core, (b) interior layers (generations, G) composed of repeating units, radially attached to the initiator core, and (c) an exterior surface of terminal functionality (i.e., terminal functional groups) attached to the outermost generation. The size and shape of the starburst dendrimer and the functional

groups present in the dendrimer can be controlled by the choice of the initiator core, the number of generations (i.e., tiers) employed in creating the dendrimer, and the choice of the repeating units employed at each generation. Since the dendrimers can be readily isolated at any particular generation, a means is provided for obtaining dendrimers having desired properties.

The choice of the starburst dendrimer components affects the properties of the dendrimers. The initiator core type can affect the dendrimer shape, producing (depending on the choice of initiator core), for example, spheroid-shaped dendrimers, cylindrical or rod-shaped dendrimers, ellipsoid-shaped dendrimers, or mushroom-shaped dendrimers. Sequential building of generations (i.e., generation number and the size and nature of the repeating units) determines the dimensions of the dendrimers and the nature of their interior.

Because starburst dendrimers are branched polymers containing dendritic branches having functional groups distributed on the periphery of the branches, they can be prepared with a variety of properties. For example, the macromolecules depicted in Figure 2A, and the starburst dendrimers, such as those depicted in Figure 2B, can have distinct properties due to branch length. The dendrimer type shown in Figure 2A possesses unsymmetrical (unequal segment) branch junctures, exterior (i.e., surface) groups (represented by Z'), interior moieties (represented by Z) but much less internal void space. The preferred dendrimer type shown in Figure 2B possesses symmetrical (equal segment) branch junctures with surface groups (represented by Z'), two

different interior moieties (represented respectively by X and Z) with interior void space which vaires as a function of the generation (G). The dendrimers such as those depicted in Figure 2B can be advanced through enough generations to totally enclose and contain void space, to give an entity with a predominantly hollow interior and a highly congested surface. Also, starburst dendrimers, when advanced through sufficient generations exhibit "starburst dense packing" where the surface of the dendrimer contains sufficient terminal moieties such that the dendrimer surface becomes congested and encloses void spaces within the interior of the dendrimer. This congestion can provide a molecular level barrier which can be used to control diffusion of materials into or out of the interior of the dendrimer.

Surface chemistry can be controlled in a predetermined fashion by selecting a repeating unit which contains the desired chemical functionality or by chemically modifying all or a portion of the surface functionalities to create new surface functionalities. In an advantageous use of the dendrimers, the dendrimers can themselves be linked together to create polydendric moieties ("starburst bridged dendrimers") which are also suitable as carriers.

In addition, the dendrimers can be prepared so as to have deviations from uniform branching in particular generations, thus providing a means of adding discontinuities (i.e., deviations from uniform branching at particular locations within the dendrimer) and different properties to the dendrimer.

The starburst polymers employed in the starburst conjugates of the present invention can be prepared according to methods known in the art, for example, U.S. Patent 4,587,329.

Dendrimers can be prepared having highly uniform size and shape and most importantly allow for a greater number of functional groups per unit of surface area of the dendrimer, and can have a greater number of functional groups per unit of molecular volume as compared to other polymers which have the same molecular weight, same core and monomeric components and same number of core branches as the starburst polymers. The increased functional group density of the starburst polymers may allow a greater quantity of material to be carried per dendrimer. Since the number of functional groups on the dendrimers can be controlled on the surface and within the interior, it also provides a means for controlling the amount of agent carried per dendrimer.

An analogy can be made between early generation starburst dendrimers (i.e. generation =1-7) to classical spherical micelles. The dendrimer-micelles analogy was derived by comparing features which they had in common such as shape, size and surface.

Table I

Parameter	Regular Classical Micelles	Starburst Dendrimers
Shape	Spherical	Spherical
Size (diameter)	20-60Å	17-67Å
Surface aggregation number	4-202	Z=6-192 (generation = 2-7)
area/surface group ($Å^2$)	$130-80Å^2$	$127-75Å^2$

Z is the number of surface groups; $1Å = 10^{-1}$ nm; $1Å^2 = 10^{-2}$ nm^2

In Table I, the shape was verified by scanning transmission electron micrographs (STEM) microscopy and intrinsic viscosity (η) measurements. The size was verified by intrinsic viscosity (η) and size exclusion chromatography (SEC) measurements. The surface aggregation numbers were verified by titremetry and high field NMR. The area/surface group was calculated from SEC hydrodynamic measurements.

The first five generations of starburst polyamidoamine (PAMAM) dendrimers are microdomains which very closely mimic classical spherical micelles in nearly every respect (i.e. shape, size, number of surface groups, and area/surface group). A major difference, however, is that they are covalently fixed and robust compared to the dynamic equilibration of nature of micelles. This difference is a significant advantage when using these microdomains as encapsulation devices.

As further concentric generations are added beyond five, congestion of the surface occurs. This congestion can lead to increased barrier characteristics at the surface and manifests itself as a smaller surface area per head (surface) groups as shown in Table II.

Table II
PAMAM Dendrimer Features vs. Generation

Generations	1	2	3	4	5	6	7	8	9
# of surface groups, Z	3	6	12	24	48	96	192	384	768
Molecular wt.	275	875	2411	5147	10,619	21,563	43,541	17,227	174,779
Diameter* measured SEC	10.4Å	15.8Å	22Å	31Å	40Å	53Å	67Å	76Å	88Å
Surface area per dendrimer	366Å^2	783Å^2	1519Å^2	3018Å^2	5024Å^2	8,820Å^2	14,096Å^2	18,136Å^2	36,083Å^2
Surface area per Z group	122Å^2	131Å^2	127Å^2	126Å^2	104Å^2	92Å^2	73Å^2	47Å^2	32Å^2
Distance between Z groups	12.4Å	12.8Å	12.7Å	12.6Å	11.5Å	10.8Å	9.8Å	7.75Å	6.28Å
Void Volume	311.6Å^3	1,470.2Å^3	4,737.9Å^3	11,427.0Å^3	---	---	---	---	---

* Hydrodynamic diameters determined by size exclusion chromatogaphy measurements calibrated against monodisperse ($\frac{Mw}{Mn}$ = 1.02) polyethyleneoxide standards.

1Å = 10^{-1} nm; 1Å^2 = 10^{-2} nm^2; 1Å^3 = 10^{-3} nm^3.

For example, amine terminated generations 5.0, 6.0, 7.0, 8.0 and 9.0 have decreased surface areas of 104, 92, 73, 47 and 32Å^2 per Z group, respectively. This characteristic corresponds to a transition from a less congested micelle-like surface to a more congested bilayer/monolayer barrier like surface normally associated with vesicles (liposomes) or Langmuir-Blodgett type membranes.

If this surface congestion is indeed occurring, the change in physical characteristics and morphology should be observed as the generations increase from the intermediate generation (6-8) to the more advanced generations (9 or 10). The scanning transmission electron micrographs (STEM) for generations = 7.0, 8.0 and 9.0 were obtained after removing the methanol solvent from each of the samples to provide colorless, light yellow solid films and staining with osmium tetraoxide. The morphological change predicted occurred at the generation G = 9.0 stage. The interior microdomains at generation, G = 9.0, measure about 33Å in diameter and are surrounded by a colorless rim which is about 25Å thick. Apparently the methanolic solvent has been entrapped within the 25Å outer membrane-like barrier to provide the dark stained interior. Thus, at generation = 9.0, the starburst PAMAM is behaving topologically like a vesicale (liposome). However, this starburst is an order of magnitude smaller and very monodispersed compared to a liposome. Consequently, the present dendrimers can be used to molecularly encapsulate solvent filled void spaces of as much diameter as about 33Å (volume about 18,000Å^3) or more.

Since the number of functional groups on the dendriers can be controlled on the surface and within

the interior, it also provides a means for controlling the amount of carried material to be delivered per dendrimer. In one embodiment of the present invention, the dendrimers are targeted carriers of agents capable of delivering the carried agent (material) to a particular locus.

Dendrimers suitable for use in the conjugates of the present invention include the starburst polymers described in U.S. Patents 4,507,466, 4,558,120, 4,568,737 and 4,587,329.

In particular, the present invention concerns a starburst conjugate which comprises at least one starburst polymer associated with at least one carried material. Starburst conjugates included within the scope of the present invention include those represented by the formula:

$$(P)_x * (M)_y \qquad (I)$$

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wherein each P represents a dendrimer;

x represents an integer of 1 or greater;

each M represents a unit (for example, a molecule, atom, ion, and/or other basic unit) of a carried material, said carried material can be the same carried material or a different carried material;

y represents an integer of 1 or greater; and

* indicates that the carried material is associated with the dendrimer.

Preferred starburst conjugates of formula (I) are those in which M is a signal generator such as fluorescing entities, signal reflector such as paramagnetic entities, signal absorbers such as electron beam opacifiers, fragrance, pheromones, or dye; particularly preferred are those in which x=1, and y=2 or more.

Also included are starburst conjugates of formula (I) wherein the dense star dendrimers are covalently linked together, optionally via linking groups, so as to form polydendric assemblages (i.e., where $x>1$).

As used herein, "associated with" means that the carried material(s) can be encapsulated or entrapped within the core of the dendrimer, dispersed partially or fully throughout the dendrimer, or attached or linked to the dendrimer, or any combination thereof. The association of the carried material(s) and the dendrimers may optionally employ connectors and/or spacers to facilitate the preparation or use of the starburst conjugates. Suitable connecting groups are groups which link a targeting director (i.e., T) to the dendrimer (i.e., P) without significantly impairing the effectiveness of the director or the effectiveness of any other carried material(s) (i.e., M) present in the starburst conjugate. These connecting groups may be cleavable or non-cleavable and are typically used in order to avoid steric hindrance between the target director and the dendrimer, preferably the connecting groups are stable (i.e., non-cleavable). Since the

size, shape and functional group density of the dense star dendrimers can be rigorously controlled, there are many ways in which the carried material can be associated with the dendrimer. For example, (a) there can be covalent, coulombic, hydrophobic, or chelation type association between the carried material(s) and entities, typically functional groups, located at or near the surface of the dendrimer; (b) there can be covalent, coulombic, hydrophobic, or chelation type association between the carried material(s) and moieties located within the interior of the dendrimer; (c) the dendrimer can be prepared to have an interior which is predominantly hollow allowing for physical entrapment of the carried materials within the interior (void space) wherein the release of the carried material can optionally be controlled by congesting the surface of the dendrimer with diffusion controlling moieties; or (d) various combinations of the aforementioned phenomena can be employed.

Dendrimers, herein represented by "P", include the dense star polymers described in U.S. Patents 4,507,466, 4,558,120, 4,568,737 or 4,587,329.

Carried materials, herein represented by "M", which are suitable for use in the starburst conjugates include any materials, other than pharmaceutical or agricultural materials, which can be associated with the starburst dendrimer without appreciably disturbing the physical integrity of the dendrimer, for example, metal ions such as the alkali and alkaline-earth metals; signal generators such as fluorescing entities; signal reflectors such as paramagnetic entities; signal absorbers such as electron beam opacifiers; pheromone moieties; fragrance moieties; dye

moieties; and the like. Carried materials include scavenging agents such as chelants or any moieties capable of selectively scavenging a variety of agents.

The starbursts conjugates of formula (I) are prepared by reacting P with M, usually in a suitable solvent, at a temperature which facilitates the association of the carried material (M) with the starburst dendrimer (P).

Suitable solvents are solvents in which P and m are at least partially miscible and inert to the formation of the conjugate. If P and M are at least partially miscible with each other, no solvent may be required. When desired, mixtures of suitable solvents can be utilized. Examples of such suitable solvents are water, methanol, ethanol, chloroform, acetonitrile, toluene, dimethylsulfoxide and dimethylformamide.

The reaction condition for the formation of the starburst conjugate of formula (I) depends upon the particular dendrimer (P), the carried material (M), and the nature of the bond (*) formed. Typically, the temperature can range from room temperature to reflux. The selection of the particular solvent and temperature will be apparent to one skilled in the art.

The ratio of M:P will depend on the size of the dendrimer and the amount of carried material. For example, the molar ratio (ratio of moles) any ionic M to P is usually 0.1-1,000:1, preferably 1-50:1 and more preferably 2-6:1. The weight ratio of any organic M to P is usually 0.1-5:1, and preferably 0.5-3:1.

Other starburst conjugates are those conjugates which contain a target director (herein designated as "T") and which are represented by the formula:

$$(T)_e * (P)_x * (M)_y \qquad \text{(II)}$$

wherein

each T represents a target director;

e represents an integer of 1 or greater; and

P, x, *, M, and y are as previously defined herein.

Preferred among the starburst conjugates of formula (II) are those in which M is a signal generator, signal reflector, or signal absorber. Also preferred are those conjugates in which e=1; and those in which x=1 and y=2 or more; and particularly preferred are those in which x=1, e=2, and y=2 or more. Most preferred are those in which M and T are associated with the polymer via the same or different connectors.

The starburst conjugates of formula (II) are prepared either by forming T*P and then adding M or by forming P*M and then adding T. Either reaction scheme is conducted at temperatures which are not detrimental to the particular conjugate component and in the presence of a suitable solvent when required. To control pH, buffers or addition of suitable acid base is used. The reaction conditions are dependent on the type of association formed (*), the starburst dendrimer used (P), the carried material (M), and the target director (T). Alternatively, P and M can be chelated,

usually in water, before conjugation to T. The conjugation with T is carried out in a suitable buffer.

The ratio of T:P is preferably 1:1. The ratio of M:P will be as before.

Target directors capable of targeting the starburst conjugates are entities which when used in the starburst conjugates of the present invention result in at least a portion of the starburst conjugates being delivered to a desired target, chemical functionalities exhibiting target specificity, and the like.

In the absence of a target director (or in the presence of a target director if desired), due to the number of functional groups which can be located at or near the surface of the dendrimer, all or a substantial portion of such functional groups can be made anionic, cationic, hydrophobic or hydrophilic to effectively aid delivery of the starburst conjugate to a desired target of the opposite charge or to a hydrophobic or hydrophilic compatible target.

Preparation of the conjugates of formula (II) using a P with a protected handle (S) is also intended as a process to prepare the conjugates of formula (II). The reaction scheme is shown below:

$$S{*}P \xrightarrow{\text{loading}} S{*}P{*}M \xrightarrow{\text{deprotection}} P{*}M \xrightarrow{\text{linking}} T{*}P{*}M$$

where

S*P represents the protected dendrimer;

S*P*M represents the protected dendrimer conjugated with m;

P*M represents the dendrimer conjugated with M (starburst conjugate);

T*P*M represents the starburst conjugates liked to the target director.

Suitable solvents can be employed which do not effect P*M. For example when S is t-butoxycarbamate, S can be removed by aqueous acid.

The starburst conjugates can be used for a variety of in vitro applications such as radioimmunoassays, electron microscopy, enzyme linked immunosorbent assays, nuclear magnetic resonance spectroscopy, and contrast imaging, and immunoscintography, in analytical applications; or used as starting materials for making other useful agents.

The present invention is also directed to starburst conjugate compositions in which the starburst conjugates are formulated with other suitable vehicles. The starburst conjugate compositions may optionally contain other active ingredients, additives and/or diluents.

The preferred starburst polymer for use in the starburst conjugates of the present invention is a polymer that can be described as a starburst polymer having at least one branch (hereinafter called a core branch), preferably two or more branches, emanating from a core, said branch having at least one terminal group provided that (1) the ratio of terminal groups to

the core branches is more than one, preferably two or greater, (2) the density of terminal groups per unit volume in the polymer is at least 1.5 times that of an extended conventional star polymer having similar core and monomeric moieties and a comparable molecular weight and number of core branches, each of such branches of the extended conventional star polymer bearing only one terminal group, and (3) a molecular volume that is no more than about 80 percent of the molecular volume of said extended conventional star polymer as determined by dimensional studies using scaled Corey-Pauling molecular models. As used herein, the term "dense" as it modifies "star polymer" or "dendrimer" means that it has a smaller molecular volume than an extended conventional star polymer having the same molecular weight. The extended conventional star polymer which is used as the base for comparison with the dense star polymer is one that has the same molecular weight, same core and monomeric components and same number of core branches as the dense star polymer. By "extended" it is meant that the individual branches of the conventional star polymer are extended or stretched to their maximum length, e.g., as such branches exist when the star polymer is completely solvated in an ideal solvent for the star polymer. In addition while the number of terminal groups is greater for the dense star polymer molecule than in the conventional star polymer molecule, the chemical structure of the terminal groups is the same.

Dendrimers used in the conjugates of the present invention can be prepared by processes known in the art. The above dendrimers, the various coreactants

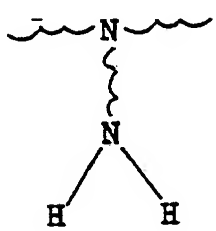
and core compounds, and process for their preparation can be as defined in U.S. Patent 4,587,329.

The starburst dendrimers, for use in the starburst conjugates of the present invention, can have terminal groups which are sufficiently reactive to undergo addition or substitution reactions. Examples of such terminal groups include amino, hydroxy, mercapto, carboxy, alkenyl, allyl, vinyl, amido, halo, urea, oxiranyl, aziridinyl, oxazolinyl, imidazolinyl, sulfonato, phosphonato, isocyanato and isothiocyanato. The dendrimers differ from conventional star or star-branched polymers in that the dendrimers have a greater concentration of terminal groups per unit of molecular volume than do conventional extended star polymers having an equivalent number of core branches and an equivalent core branch length. Thus, the density of terminal groups per unit volume in the dendrimer usually is at least about 1.5 times the density of terminal groups in the conventional extended star polymer, preferably at least 5 times, more preferably at least 10 times, most preferably from 15 to 50 times. The ratio of terminal groups per core branch in the starburst dendrimer is preferably at least 2, more preferably at least 3, most preferably from 4 to 1024. Preferably, for a given polymer molecular weight, the molecular volume of the starburst dendrimer is less than 70 volume percent, more preferably from 16 to 60, most preferably from about 7 to 50 volume percent of the molecular volume of the conventional extended star polymer.

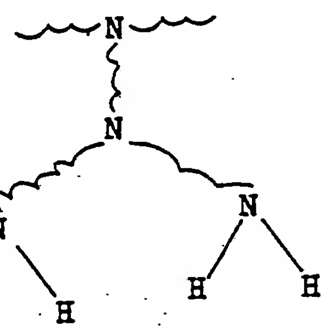
Preferred starburst dendrimers for use in the starburst conjugates of the present invention are characterized as having a univalent or polyvalent core

that is covalently bonded to dendritic branches. Such ordered branching can be illustrated by the following sequence wherein G indicates the number of generations:

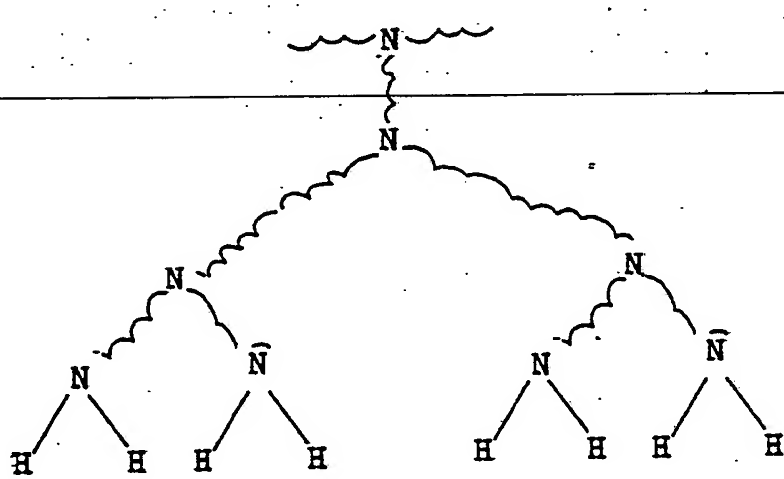
G = 1



G = 2



G = 3



Mathematically, the relationship between the number (#) of terminal groups on a dendritic branch and the number of generations of the branch can be represented as follows:

$$\text{\# of terminal groups per dendritic branch} = \frac{N_r^G}{2}$$

wherein G is the number of generations and N_r is the repeating unit multiplicity which is at least 2 as in the case of amines. The total number of terminal groups in the dendrimer is determined by the following:

$$\text{\# of terminal groups per dendrimer} = \frac{N_c N_r^G}{2}$$

wherein G and N_r are as defined before and N_c represents the valency (often called core functionality) of the core compound. Accordingly, the dendrimers of this invention can be represented in its component parts as follows:

$$(\text{Core})\left\{(\text{Repeat Unit})_{\frac{N_r^G - 1}{N_r - 1}} \begin{matrix}\text{Terminal} \\ \text{Moiety}\end{matrix}_{N_r^G}\right\}_{N_c}$$

wherein the Core, Terminal Moiety, G and N_c are as defined before and the Repeat Unit has a valency or functionality of $N_r + 1$ wherein N_r is as defined before.

A copolymeric dendrimer which is a preferred dendrimer for the purposes of this invention is a unique compound constructed of polyfunctional monomer units in a highly branched (dendritic) array. The dendrimer molecule is prepared from a polyfunctional initiator unit (core compound), polyfunctional repeating units and terminal units which may be the same or different from the repeating units. The core compound is represented by the formula I $(Z^c)_{Nc}$ wherein I represents the core, Z^c represents the functional groups bonded to I and Nc represents the core functionality which is preferably 2 or more, most preferably 3 or more. Thus, the dendrimer molecule comprises a polyfunctional core, I bonded to a number (N_c) of functional groups, Z^c, each of which is connected to the monofunctional tail of a repeating unit, $X^1Y^1(Z^1)_{N^1}$, of the first generation and each of the Z groups of the repeating unit of one generation is bonded to a monofunctional tail of a repeating unit of the next generation until the terminal generation is reached.

In the dendrimer molecule, the repeating units are the same within a single generation, but may differ from generation to generation. In the repeating unit, $X^1Y^1(Z^1)_{N^1}$, X^1 represents the monofunctional tail of the first generation repeating unit, Y^1 represents the moiety constituting the first generation, Z^1 represents the functional group of the polyfunctional head of the repeating unit of the first generation and may be the

same as or different from the functional groups of the core compound, Ⓘ$(Z^c)_{Nc}$, or other generations; and N^1 is a number of 2 or more, most preferably 2, 3 or 4, which represents the multiplicity of the polyfunctional head of the repeating unit in the first generation. Generically, the repeating unit is represented by the formula $X^iY^i(Z^i)_{N^i}$ wherein "i" represents the particular generation from the first to the t-1 generation. Thus, in the preferred dendrimer molecule, each Z^1 of the first generation repeating unit is connected to an X^2 of a repeating unit of the second generation and so on through the generations such that each Z^i group for a repeating unit $X^iY^i(Z^i)_{N^i}$ in generation number "i" is connected to the tail (X^{i+1}) of the repeating unit of the generation number "i+1". The final or terminal of a preferred dendrimer molecule comprises terminal units, $X^tY^t(Z^t)_{N^t}$ wherein t represents terminal generation and X^t, Y^t, Z^t and N^t may be the same as or different from X^i, Y^i, Z^i and N^i except that there is no succeeding generation connected to the Z^t groups and N^t may be less than two, e.g., zero or one. Therefore the preferred dendrimer has a molecular formula represented by

$$\left(\text{(I)}(Z^c)_{N_c}\right)\left\{\left(X^i\ Y^i\ (Z^i)_{N^i}\right)_{N_c \prod_{n \text{ is } 1}^{i-1} N^n}\right\}\left(X^tY^t(Z^t)_{N^t}\right)_{N_c \prod_{n \text{ is } 1}^{t-1} N^n}$$

where i is 1 to t-1

wherein the symbols are as previously defined. The Π function is the product of all the values between its defined limits. Thus

$$\prod_{n=1}^{i-1} N^n = (N^1)(N^2)(N^3)\cdots(N^{i-2})(N^{i-1})$$

which is the number of repeat units, $X^iY^i(Z^i)_{N^i}$, comprising the ith generation of one dendritic branch and when i is 1, then

$$\prod_{n=1}^{o} = 1 \quad .$$

In copolymeric dendrimers, the repeat unit for one generation differs from the repeat unit in at least one other generation. The preferred dendrimers are very symmetrical as illustrated in structural formulas described hereinafter. Preferred dendrimers may be converted to functionalized dendrimers by contact with another reagent. For example, conversion of hydroxyl in the terminal generation to ester by reaction with an acid chloride gives an ester terminally functionalized dendrimer. This functionalization need not be carried out to the theoretical maximum as defined by the number

of available functional groups and, thus, a functionalized dendrimer may not have high symmetry or a precisely defined molecular formula as is the case with the preferred dendrimer.

In a homopolymeric dendrimer, all of the repeat units, $X^iY^i(Z^i)_{N^i}$, are identical. Since the values of all N^i are equal (defined as N_r), the product function representing the number of repeat units reduces to a simple exponential form. Therefore, the molecular formula may be expressed in simpler form as

$$\left(\text{I}(Z^c)_{N_c}\right)\left\{\left(X^i\ Y^i\ (Z^i)_{N^i}\right)_{N_cN_r^{i-1}}\right\}\left(X^tY^t(Z^t)_{N^t}\right)_{N_cN_r^{(t-1)}}$$

where i = 1 to t-1

This form still shows the distinction between the different generations i, which each consist of $N_cN_r^{(i-1)}$ repeating units, $X^iY^i(Z^i)_{N^i}$. Combining the generations into one term gives:

$$\left(\text{(I)}(Z^c)_{N_c}\right)\left(X^iY^i(Z^i)_{N_r}\right)_{N_c\frac{N_r^{(t-1)}-1}{N_r-1}}\left(X^tY^t(Z^t)_{N^t}\right)_{N_cN_r^{(t-1)}}$$

or

core repeat unit terminal unit,

$$\left(\text{(I)}(Z^c)_{N_c}\right)\left\{\left(X^rY^r(Z^r)_{N_r}\right)_{\frac{N_r^{(t-1)}-1}{N_r-1}}\left(X^tY^t(Z^t)_{N^t}\right)_{N_r^{(t-1)}}\right\}_{N_c}$$

wherein $X^rY^r(Z^r)_{N_r}$ is the repeating unit which is used in all generations i.

Consequently, if a polymer compound will fit into these above formulae, then the polymer is a starburst polymer. Conversely, if a polymer compound will not fit into these above formulae, then the polymer is not a starburst polymer. Also, to determine whether a polymer is a starburst polymer, it is not necessary to know the process by which it was prepared, but only whether it fits the formulae. The formulae also demonstrate the generations (G) or tiering of dendrimers.

Clearly, there are several ways to determine the ratio of agent (M) to dendrimer (P) which depend upon how and where the association of P*M occurs. When there is interior encapsulation, the weight ratio of

M:P usually is 10:1, preferably 8:1, more preferably 5:1, most preferably 3:1. The ratio can be as low as 0.5:1 to 0.1:1. When interior stoichiometry is used, the weight ratio of M:P is the same as for interior encapsulation. When exterior stoichiometry is determined, the mole/mole ratio of M:P given by the following formulae:

	M	:	P
(A)	$5\ N_cN_tN_r^{G-1}$		1
(B)	$3\ N_cN_tN_rG^{-1}$		1
(C)	$1\ N_cN_tN_r^{G-1}$		1

where N_c means the core multiplicity, N_t means the terminal group multiplicity, and N_r means branch juncture multiplicity. The $N_cN_tN_rG^{-1}$ term will result in the number of Z groups. Thus, for example, (A) above may result when proteins, enzymes or highly charged molecules are on the surface; (B) above when it is octanoic acid; (C) above when converting surface ester groups to carboxylate ions or groups.

Of course other structures of various dimensions can be readily prepared by one skilled in the art by appropriately varying the dendrimer components and number of generations employed. The dimensions are significant in that they are small. A linear polymer of comparable molecular weight would have a radius of gyration, (in its fully extended form), that would be much larger than the same molecular weight dendrimer.

Linking target directors to dendrimers is another aspect of the present invention. In preferred embodiments of the present invention, a reactive functional group such as a carboxyl, sulfhydryl, reactive aldehyde, reactive olefinic derivative, isothiocyanato, isocyanato, amino, reactive aryl halide, or reactive alkyl halide can conveniently be employed on the dendrimer. The reactive functional groups can be introduced to the dendrimer using known techniques, for example:

(1) Use of a heterofunctional initiator (as a starting material for synthesizing the dendrimer) which has incorporated into it functional groups of different reactivity. In such heterofunctional initiator at least one of the functional groups will serve as an initiation site for dendrimer formation and at least one of the other functional groups will be available for linking to a target director but unable to initiate dendrimer synthesis. For example, use of protected aniline to allow further modification of NH_2 groups within the molecule without reacting the aniline NH_2.

The functional group which will be available for linking to a target director may be part of the initiator molecule in any one of three forms, namely:

(a) In the form in which it will be used for linking with the target directors. This is possible when none of the synthetic steps involved in the dendrimer synthesis can result in reaction at this center

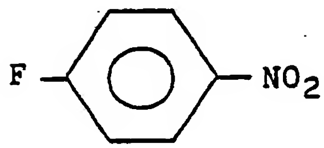
(b) When the functional group used for linking to the targeting director is reactive in

the synthetic steps involved in the dendrimer synthesis, it can be protected by use of a protecting group, which renders the group unreactive to the synthetic procedures involved, but can itself be readily removed in a manner which does not alter the integrity of the remainder of the macromolecule.

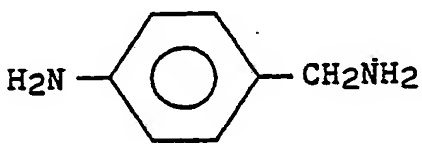
(c) In the event that no simple protecting group can be found for the reactive functionality to be used for linking with the targeting director, a synthetic precursor can be used which is unreactive in all the synthetic proecedures used in the dendrimer synthesis. On completion of the synthesis, this functional group must be readily convertible into the desired linking group in a manner which does not alter the integrity of the remainder of the molecule.

(2) Coupling (covalently) the desired reactive functional group onto a preformed dendrimer. The reagent used must contain a functionality which is readily reacted with the terminal functional groups of the dendrimer. The functional group to be ultimately used to link with the targeting agent can be in its final form, as a protected functionality, or as a synthetic precursor. The form in which this linking functionality is used depends on its integrity during the synthetic procedure to be utilized, and the ability of the

final macromolecule to withstand any conditions necessary to make this group available for linking. For example, the preferred route for PEI uses



Examples of heterofunctional initiators for use in (1) above, include the following illustrative examples:



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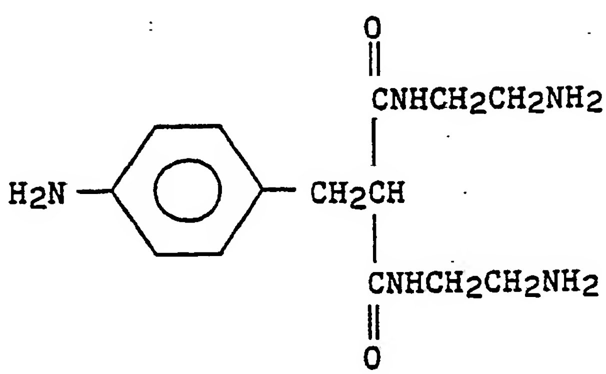

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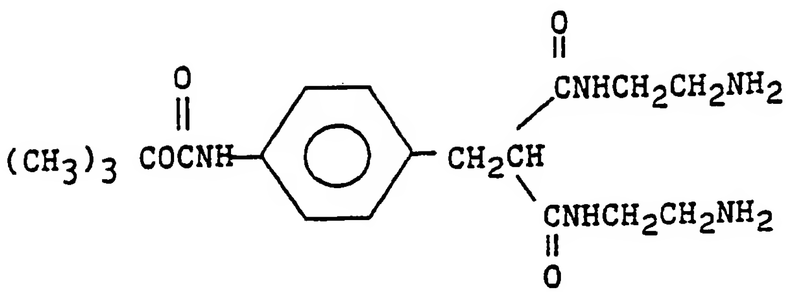
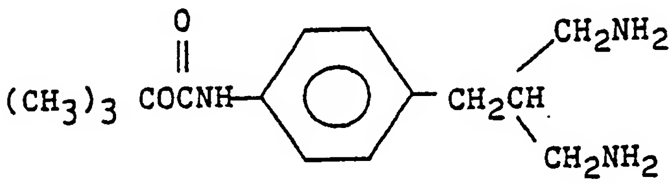
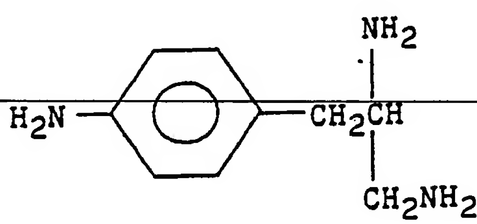

O
CNHCH2CH2NH2
O
(CH3)3 COCNH
CH2CH
CNHCH2CH2NH2
O

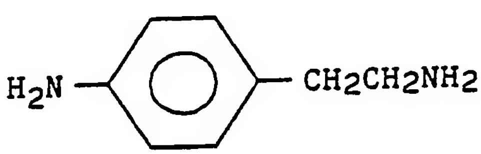
;


O
CH2NH2
(CH3)3 COCNH
CH2CH
CH2NH2

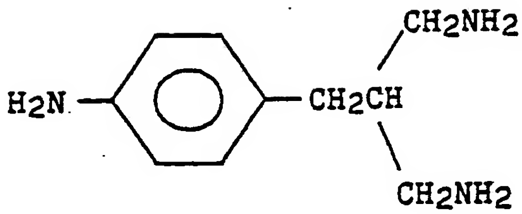
;


NH2
H2N
CH2CH
CH2NH2

;


H2N
CH2CH2NH2

;



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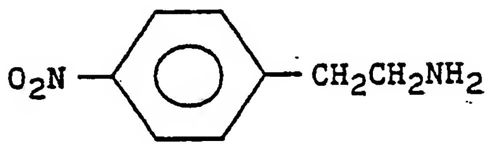

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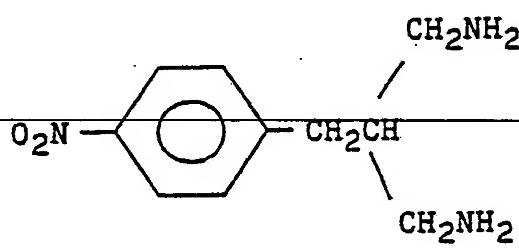
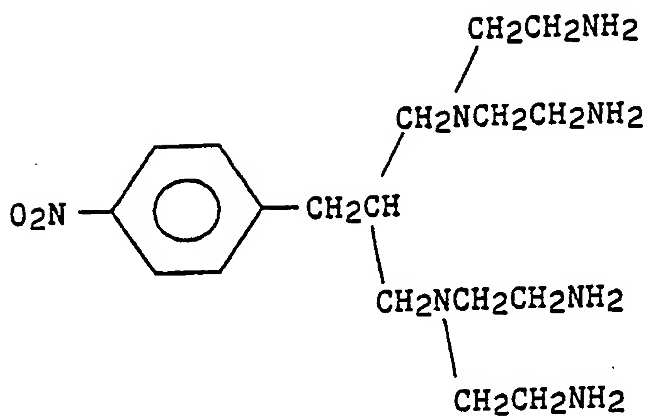


; and



There are several chemistries of particular importance:

1) Starburst Polyamidoamides ("PAMAM") Chemistry;

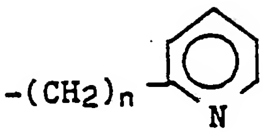
2) Starburst Polyethyleneimines ("PEI") Chemistry;

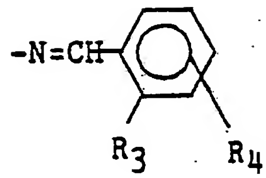
3) Starburst PEI compound with a surface of PAMAM;

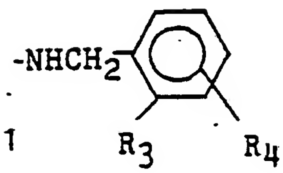
4) Starburst Polyether ("PE") Chemistry.

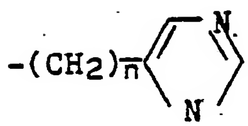
Modifications of the dendrimer surface functionalities may provide other useful functional groups such as the following:

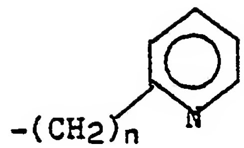
$-OPO_3H_2$, $-PO_3H_2$, $-PO_3H^{(-1)}$, $-PO_3^{(-2)}$, $-CO_2^{(-1)}$, $-SO_2H$, $-SO_2^{(-1)}$, $-SO_3H$, $-SO_3^{(-1)}$, $-NR^1R^2$, $-R^5$, $-OH$, $-OR^1$, $-NH_2$, polyethers, perfluorinated alkyl, $-\underset{\underset{O}{\|}}{C}NHR^1$, $-\underset{\underset{O}{\|}}{C}OH$,

,

,

,

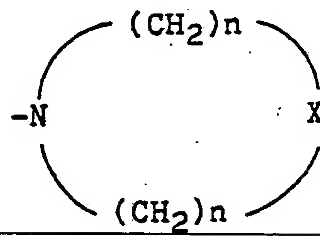
,



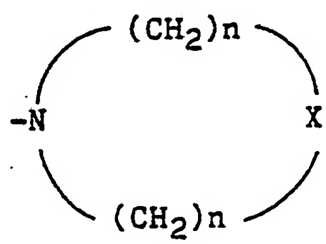
wherein

R represents alkyl, aryl or hydrogen;

R^1 represents alkyl, aryl, hydrogen, or

;

R^2 represents alkyl, aryl, or

;

$R3$ represents $-OH$, $-SH$, $-CO_2H$, $-SO_2H$, or $-SO_3H$;

R^4 represents alkyl, aryl, alkoxy, hydroxyl, mercapto, carboxyl, nitro, hydrogen, bromo, chloro, iodo, or fluoro;

$R5$ represents alkyl;

x represents NR, O or S; and

n represents the integer 1, 2 or 3.

The choice of functional group depends upon the particular end use for which the dendrimer is designed.

The following examples further illustrate the invention but are not to be construed as a limitation on the scope of the invention. The lettered examples concern the preparation of starting materials; the numbered examples concern the preparation of product.

Example A: Preparation of 2-Carboxamido-3-(4'-nitrophenyl)-propanamide.

p-Nitrobenzyl malonate diethylester (2.4 grams (g), 8.13 mmole) was dissolved in 35 ml of methanol. The solution was heated to 50-55°C with stirring and a stream of anhydrous ammonia was bubbled through the solution for 64 hours. The solution was cooled and the white, flocculant product was filtered and recrystallized from 225 milliliters (ml) of boiling methanol to afford 1.85 g (7.80 mmole) of bis amide in 96% yield (mp = 235.6°C(d)).

The structure was confirmed by MS, ^1H and ^{13}C NMR spectroscopy.

Anal: Calc. for $C_{10}H_{11}O_4N_3$

	C	H	N
Theo:	50.63	4.69	17.72
Found:	50.75	4.81	17.94

Example B: Preparation of 1-Amino-2-(aminomethyl)-3-(4'-nitrophenyl)propane.

2-Carboxamido-3-(4'nitrophenyl)propanamide (2.0 g, 8.43 mmole) was slurried in 35 ml of dry tetrahydrofuran under a nitrogen atmosphere with stirring. To this mixture was added borane/tetrahydrofuran complex (106 ml, 106 mmole) via syringe. The reaction mixture was then heated to reflux for 48 hours during which time the suspended amide dissolved. The solution was cooled and the tetrahydrofuran was removed *in vacuo* using a rotary evaporator. The crude product and borane residue was dissolved in 50 ml of ethanol and this solution was purged with anhydrous hydrogen chloride gas. The solution was refluxed for 1 hour and the solvent removed *in vacuo*. The crude hydrochloride salt was dissolved in 15 ml of deionized water and extracted with two 50 ml portions of methylene chloride. The aqueous layer was cooled in an ice bath under an argon blanket and 50% sodium hydroxide was slowly added until basic pH=11.7. The basic aqueous layer was extracted with four 25 ml portions of methylene chloride and these combined extracts were evaporated (rotary) to give 1.45 g of amber colored oil. This oil was triturated with diethyl ether (50 ml) and filtered under pressure through a short silica gel (grade 62 Aldrich) column. The column was washed with 100 ml of ether and the combined filtrates were vacuum evaporated giving 1.05 g (5.02 mmole) of the

titled diamine as a clear oil (mp = 275-278°C(d) bis HCl salt).

The structure was confirmed by MS, ^{1}H and ^{13}C NMR spectroscopy.

<u>Anal:</u> Calc. for $C_{10}H_{17}N_3O_2Cl_2$

	C	H	N
Theo:	42.57	6.07	14.89
Found:	43.00	6.14	15.31

<u>Example C:</u> Preparation of 1-Amino-2-(aminomethyl)-3-(4'-aminophenyl)propane.

Borane/tetrahydrofuran solution (70 ml, 70 mmole) was added under nitrogen *via* a cannula needle to a flask containing 4-amino-benzyl malonamide (1.5 g, 7.24 mmole) with stirring. The solution was brought to reflux for 40 hours. The colorless solution was cooled and excess tetrahydrofuran was removed by rotary evaporation leaving a clear gelatinous oil. Methanol (50 ml) was cautiously added to the oil with notable gas evolution. Dry hydrogen chloride was bubbled through the suspension to effect dissolution and the solution was then refluxed for 1 minute. The methanol/HCl was rotary evaporated and the resulting hydrochloride salt was carried through the same dissolution/reflux procedure again. The hydrochloride salt obtained was dissolved in 10 ml of water and cooled in an ice bath under argon. Concentrated sodium hydroxide (50%) was added slowly with stirring to pH=11. The aqueous portion was then extracted with 2 X 100 ml portions of chloroform which were combined and filtered through a short silica gel plug without drying. The solvent was removed *in vacuo* (rotary)

affording the title compound (0.90 g, 5.02 mmole) in 70% yield (R_f=0.65 - $CHCl_3$/MeOH/NH_4OH conc - 2/2/1). The structure was confirmed by ^1H and ^{13}C NMR and used without further purification.

Example D: Preparation of 6-(4-Aminobenzyl)-1,4,8,11-tetraaza-5,7-dioxoundecane.

4-Aminobenzyl malonate dimethylester (2.03 g, 8.43 mmole) was dissolved in 10 ml of methanol. This solution was added dropwise to a stirred solution of freshly distilled ethylene diamine (6.00 g, 103.4 mmole) in 10 ml of methanol under nitrogen over a 2 hour period. The clear solution was stirred for 4 days and thin layer chromotography (TLC) analysis indicated total conversion of diester (R_f = 0.91) to the bis amide (R_f = 0.42 - 20% conc NH_4OH/80% ethanol). This material was strongly ninhydrin positive. The methanol and excess diamine were removed on a rotary evaporator and the resulting white solid was vacuum dried (10^{-1} mm, 50°C) overnight to afford crude product (2.45g, 8.36 mmole) in 99% yield. An analytical sample was recrystallized from chloroform/hexane, MP = 160-161°C. The mass spectral, ^1H and ^{13}C NMR data were consistent with the proposed structure.

Example E: Reaction of Mesyl Aziridine with 1-Amino-2-(aminomethyl)-3-(4-nitrophenyl)propane.

1-Amino-2-(aminomethyl)-3-(4-nitrophenyl)-propane (400 mg, 1.91 mmole, >96% pure) was dissolved in 10.5 ml of absolute ethanol under nitrogen. Mesyl aziridine (950 mg, 7.85 mmole) was added to the stirred diamine solution as a solid. The reaction was stirred at 25°C for 14 hours using a magnetic stirrer and during

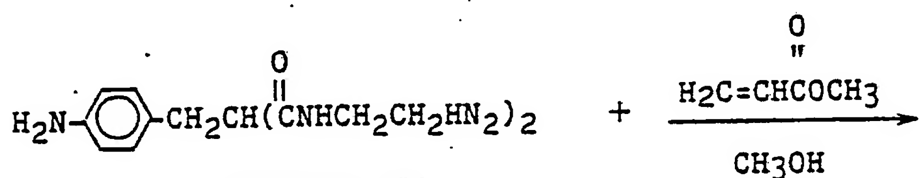
this period a white, gummy residue formed on the sides of the flask. The ethanol was decanted and the residue was triturated with another 15 ml portion of ethanol to remove any unreacted aziridine. The gummy product was vacuum dried (10^{1}mm, 25°C) to afford the tetrakis methyl sulfonamide (1.0 g, 1.44 mmole) in 75% yield (R_f = 0.74 - NH_4OH/ethanol - 20/80). The structure was confirmed by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy.

Example F: Preparation of 2-(4-Nitrobenzyl)-1,3-(bis-N,N-2-aminoethyl)diaminopropane.

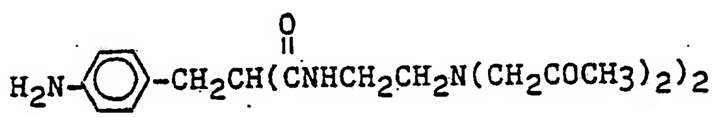
The crude methylsulfonamide (650 mg, 0.94 mmole) was dissolved in 5 ml of nitrogen purged, concentrated sulfuric acid (98%). This solution was maintained under nitrogen and heated to 143-146°C for 27 minutes with vigorous stirring. A slight darkening was noted and the cooled solution was poured into a stirred solution of ether (60 ml). The precipitated white salt cake was filtered and immediately dissolved in 10 ml of deionized water. The pH of the solution was adjusted to pH=11 with 50% NaOH under argon with cooling. The resulting solution was mixed with 90 ml of ethanol and the precipitated inorganic salts were filtered. The solvent was removed from the crude amine under reduced pressure and to the resulting light brown oil was added 190 ml of toluene under nitrogen. The mixture was stirred vigorously and water was removed through azeotropic distillation (Dean-Stark trap) until the remaining toluene acquired a light yellow color (30-40 ml remaining in pot). The toluene was cooled and decanted from the dark, intractable residues and salt. This solution was stripped of solvent *in vacuo* and the resulting light yellow oil was vacuum dried (0.2 mm,

35°C) overnight affording 210 mg of the product (60%) which was characterized by MS, ^{1}H and ^{13}C NMR.

Example G: Preparation of a starburst polymer (containing an aniline derivative) of one half generation represented by the following scheme:



Compound #1



Compound #2

Methyl acrylate (2.09 g, 24 mmole) was dissolved in methanol (15 ml). The compound 6-(4-aminobenzyl)-1,4,8,11-tetraaza-5,7-dioxoundecane (1.1 g, 3.8 mmole) (i.e., Compound #1) was dissolved in methanol (10 ml) and was added slowly over 2 hours with rigorous stirring to the methyl acrylate solution. The reaction mixture was stirred for 48 hours at ambient temperatures. The solvent was removed on the rotary evaporator maintaining the temperature below 40°C. The ester (Compound #2) was obtained as a yellow oil (2.6 g). No carboxyethylation of the aniline function was observed.

Example H: Preparation of a starburst polymer (containing an aniline moiety) of one generation; represented by the following scheme:

$$\text{Compound \#2} \quad + \quad \xrightarrow[CH_3OH]{H_2NCH_2CH_2NH_2}$$

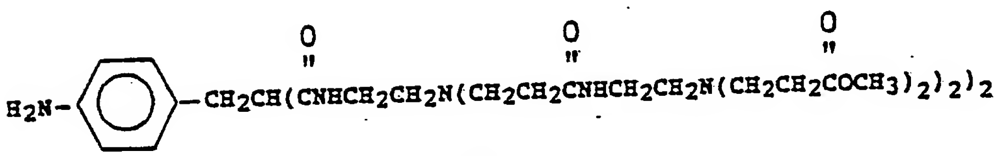
$$H_2N\text{-}C_6H_4\text{-}CH_2CH(\overset{O}{\overset{\|}{C}}NHCH_2CH_2N(CH_2CH_2\overset{O}{\overset{\|}{C}}NHCH_2CH_2NH_2)_2)_2$$

Compound #3

The ester (Compound #2) (2.6 g, 3.7 mmole) was dissolved in methanol (100 ml). This was carefully added to a vigorously stirring solution of ethylene diamine (250 g, 4.18 mole) and methanol (100 ml) at such a rate that the temperature did not rise above 40°C. After complete addition the reaction mixture was stirred for 28 hours at 35-40°C (heating mantle). After 28 hours no ester groups were detectable by infrared specroscopy. The solvent was removed on the rotary evaporator at 60°C. The excess ethylene diamine was removed using a ternary azeotrope of toluene-methanol-ethylene diamine. Finally all remaining toluene was azeotroped with methanol. Removal of all the methanol yielded 3.01 g of the product (Compound #3) as an orange glassy solid.

<u>Example I:</u> Preparation of a starburst polymer (containing an aniline moiety) of one and one half generations represented by the following scheme:

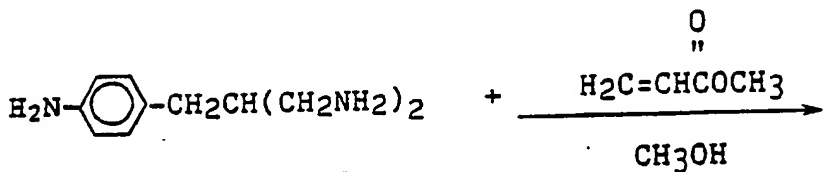
$$\text{Compound \#3} + \xrightarrow[CH_3OH]{H_2C{=}CHC(=O)OCH_3}$$



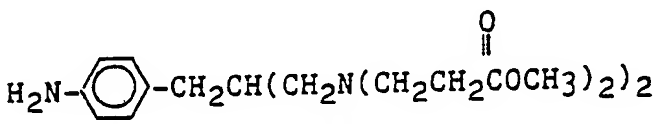
Compound #4

The amine (Compound #3) (2.7 g, 3.6 mmole) was dissolved in methanol (7 ml) and was added slowly over one hour to a stirred solution of methyl acrylate (3.8 g, 44 mmole) in methanol (15 ml) at ambient temperatures. A slight warming of the solution was observed during the addition. The solution was allowed to stir at ambient temperatures for 16 hours. The solvent was removed on the rotary evaporator at 40°C. After removal of all the solvent and excess methyl acrylate the ester (Compound #4) was obtained in 4.7 g yield as an orange oil.

Example J: Preparation of a starburst polymer (containing an aniline moiety) of one half generation represented by the following scheme:



Compound #5



Compound #6

The triamine (Compound #5, the preparation of this compound is shown in Example C) (0.42 g, 2.3 mmole) was dissolved in methanol (10 ml) and was added dropwise over one hour to methyl acrylate (1.98 g, 23 mmole) in methanol (10 ml). The mixture was allowed to stir at ambient temperatures for 48 hours. The solvent was removed on the rotary evaporator, maintaining the temperature at no higher than 40°C. The excess methyl acrylate was removed by repeated azeotroping with methanol. The ester (Compound #6) was isolated as an orange oil (1.24 g).

<u>Example K:</u> Preparation of a starburst polymer (containing an aniline moiety) of one generation; represented by the following scheme:

$$\text{Compound \#6} \quad + \quad \xrightarrow[CH_3OH]{H_2NCH_2CH_2NH_2}$$

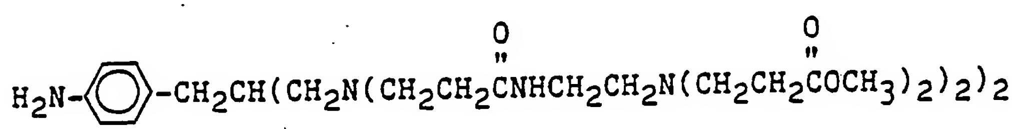
$$H_2N-C_6H_4-CH_2CH(CH_2N(CH_2CH_2\overset{O}{\overset{\|}{C}}NHCH_2CH_2NH_2)_2)_2$$

Compound #7

The ester (Compound #6) (1.24 g, 2.3 mmole) was dissolved in methanol (50 ml) and was added dropwise over two hours to ethylenediamine (73.4 g, 1.22 mole) in methanol (100 ml). A small exotherm was noted, vigorous stirring was maintained. The solution was left to stir at ambient temperatures for 72 hours. The solvent was removed on the rotary evaporator at 60°C. The excess ethylene diamine was removed using a ternary azeotrope of toluene-methanol-ethylenediamine. Finally all remaining toluene was removed with methanol and then pumping down with a vacuum pump for 48 hours gave the amine (Compound #7) (1.86 g) as a yellow/orange oil.

Example L: Preparation of a starburst polymer (containing an aniline moiety) of one and one half generations; represent by the following scheme:

$$\text{Compound \#7} \quad + \quad \xrightarrow[\text{CH}_3\text{OH}]{\text{H}_2\text{C=CHC(=O)OCH}_3}$$



Compound #8

The amine (Compound #7) (1.45 g, trace of methanol remained) was dissolved in methanol (100 ml) and was added slowly over 1½ hours to a stirred solution

of methyl acrylate (5.80 g) in methanol (20 ml). The solution was allowed to stir for 24 hours at room temperature. Removal of the solvent followed by repeated azeotroping with methanol enabled the removal of all the excess methyl acrylate. After pumping down on a vacuum pump for 48 hours the ester (Compound #8) was isolated as an orange oil (2.50 g; 1.8 mmole).

Example M: Hydrolysis of 4.5 generation dendrimer and preparation of calcium salt.

4.5 Generation PAMAM (ester terminated, initiated off NH3) (2.11 g, 10.92 meq) was dissolved in 25 ml of methanol and to it was added 10% NaOH (4.37 ml, 10.92 meq) (pH = 11.5-12). After 24 hours at room temperature, the pH was about 9.5. After an additional

20 hours, the solution was rotovaped, 50 ml of toluene added, and rotovaped again.

The resulting oil was dissolved in 25 ml of methanol and precipitated as a white gum upon addition of 75 ml of diethyl ether. The liquid was decanted, and the gum was rotovaped to give a very fine off-white powder which upon drying gives 2.16 g of product (98% yield). No ester groups were found upon NMR and infrared analysis.

The sodium salt of 4.5 Generation PAMAM (ester terminated, initiated from NH_3) was replaced by the calcium via dialysis. The sodium salt (1.03 g) was dissolved in 100 ml of water and passed through hollow fiber dialysis tubing (cut off = 5000) at 3 ml/minute. The exterior of the tubing was bathed in 5% $CaCl_2$ solution. This procedure was then repeated.

The resulting solution was again dialyzed, this time against water, then repeated two additional times.

Evaporation provided 0.6 g of wet solid, which was taken up in methanol (not totally soluble) and is dried to give 0.45 g of off-white crystals.

$C_{369}H_{592}O_{141}N_{91}Ca_{24}$ Calc. - 10.10% Ca^{++}

M Wt. = 9526.3 Calc. = C-4432.1, H-601.8, O-2255.9, N-1274.6, Ca-961.9)

Theo: C-46.5, H-6.32, N-13.38, Ca-10.10
Found: C-47.34, H-7.00, N-13.55, Ca-8.83

Example N: Preparation of dendrimers with terminal carboxylate groups.

Half-generation starburst polyamidoamines were hydrolyzed to convert their terminal methyl ester groups to carboxylates. This generated spheroidal molecules with negative charges dispersed on the periphery. The dendrimers hydrolyzed ranged from 0.5 generation (three carboxylates) to 6.5 generation (192 carboxylates).

The products could be generated as Na^+, K^+, Cs^+ or Rb^+ salts.

Example O: N-t-butoxycarbonyl-4-aminobenzyl malonate dimethylester

4-Aminobenzyl malonate dimethylester (11.62 g, 49 mmol) was dissolved in 50 ml of 5-butanol:water 60:40 with stirring. Di-t-butoxydicarbonate (19.79g, 90 mmol) was added and the reaction mixture stirred overnight. The butanol was removed on the rotary evaporator, resulting in a yellow suspension of the product in water. Extraction into methylene chloride, drying ($MgSO_4$) and evaporation gave a yellow oil (21.05 g, contaminated by di-t-butoxydicarbonate). recrystallization from 2-propanol:water (75:25) yield pale yellow crystals (11.1 g, 33 mmol, 67%). The structure was confirmed by 13_C NMR and purity checked by hplc analysis (spherisorb ODS-1, 0.05M H_3PO_4 pH 3: CH_3CN 55:45). The material was used without further purification.

Example P: N-t-butoxycarbonyl-6-(4-aminobenzyl)-1,4,8,11-tetraaza-5,7-dioxoundecane

N-t-butoxycarbonyl-4-aminobenzyl malonate dimethylester (8.82 g 26 mmol), prepared in Example O, was dissolved in 50 ml of methanol, This solution was added dropwise (2 hours) to a solution of freshly distilled ethylenediamine (188 g 3.13 mole) and 20 ml of methanol, under a nitrogen atmosphere. The solution was allowed to stir for 24 hours. The ethylene diamine/methanol solution was removed on the rotary evaporator. The product was dissolved in methanol and toluene added. Solvent removal on the rotary evaporator gave the crude product as a white solid (10.70 g contaminated with ethylenediamine). The sample was divided into two samples for purification. Azeotropic removal of ethylenediamine with toluene, using a soxhlet extractor with sulphonated ion exchange beads in the thimble to trap the ethylenediamine, resulted in partial decomposition of the product, giving a brown oil. The remaining product was isolated as a white solid from the toluene on cooling (2.3 g approximately 50 percent). Analysis of a 10 percent solution in methanol by gas chromatography (Column, Tenax 60/80) showed no ethylenediamne detectable in the sample (<0.1 percent). The second fraction was dissolved in methanol to give a 10 percent solution (by weight) and purified from the ethylenediamine by reverse osmosis, using methanol as the solvent. (The membrane used was a Filmtec™ FT-30 , in an Amicon TC1R thin channel separator, the ethylenediamine crossing the membrane.) The product was isolated as a white solid (2.7 g), in which no detectable amounts of ethylenediamine could be found by gas chromatography. The ^{13}C NMR data and hplc analysis (Spherisorb ODS-1,

0.05M H_3PO_4 pH 3:CH_3CN 55:45) were consistent with the proposed structure. The product was used with no further purification.

Example Q: Preparation of a starburst dendrimer of one half generation from N-t-butoxycarbonyl-6-(4-aminobenzyl)-1,4,8,11-tetraaza-5,7-dioxoundecane

N-t-butoxycarbonyl-6-(4-aminobenzyl)-1,4,8,11-tetraaza-5,7-dioxoundecane (5.0 g 13 mmol), prepared in Example P, was dissolved in 100 ml of methanol. Methyl acrylate (6.12 g, 68 mmol) was added and the solution stirred at ambient temperatures for 72 hours. The reaction was monitored by HPLC (Spherisorb ODS1, Acetonitrile: 0.04M Ammonium acetate 40:60) to optimize conversion to the desired product. The solution was concentrated to 30 percent solids, and methyl acrylate (3.0 g 32 mmol) was added. The reaction mixture was stirred at ambient temperatures until no partially alkylated products were detectable by HPLC (24 hours). Removal of the solvent at 30°C by rotary evaporation, and pumping down at 1 mm Hg for 24 hours gave the product as yellow viscous oil, yield 7.81 g. The 13C NMR data was consistent with the proposed structure. The product was used without further purification.

Example R: Preparation of a starburst dendrimer of one full generation from N-t-butoxycarbonyl-6-(4-aminobenzyl)-1,4,8,11-tetraaza-5,7-dioxoundecane

The half generation product (Example Q) (7.70 g, 10.45 mmol) was dissolved in 75 ml of methanol and added dropwise over 2 hours to a stirred solution of ethylenediamine (400 ml, 7.41 mol) and methanol (50 ml). The reaction mixture was stirred at ambient temperatures for 48 hours. The ethylenediamine and

methanol were removed by rotary evaporation to give a yellow oil (11.8 g contaminated with ethylene diamine). The product was dissolved in 90 ml of methanol, and purified from the ethylenediamine by reverse osmosis (Filmtec FT-30 membrane and Amicon TC1R thin channel separator, methanol as solvent). After 48 hours, no ethylenediamine could be detected by gas chromatography (Column, Tenax 60/80). Removal of the solvent on the rotary evaporator, followed by pumping down on a vacuum line for 24 hours gave the product as a yellow glassy solid (6.72 g). Analysis by HPLC, PLRP-S column, acetonitrile:0.015M NaOH, 10-20 percent gradient in 20 min.) and 13C NMR analysis was consistent with the proposed structure.

Example S: Preparation of a starburst polymer of one and one half generation from N-t-butoxycarbonyl-6-(4-aminobenzyl)-1,4,8,11-tetraaza-5,7-dioxoundecane

The one generation product (Example R) (2.14 g, 25 mmol) was dissolved in 12.5 ml of methanol, and methyl acrylate (3.5 g, 39 mmol) in 5 ml of methanol was added. The solution was stirred at ambient temperatures for 48 hours, monitoring the progress of the reaction by HPLC (Spherisorb ODS-1, acetonitrile: 0.04M ammonium acetate, 60:40). A second aliquot of methyl acrylate was added (3.5 g 39 mmol) and the reaction mixture stirred at ambient temperatures for a further 72 hours. Removal of the solvent on the rotary evaporator gave the product as a yellow oil (3.9 g) after pumping down overnight with a vacuum pump. The product was used with no further purification.

Example T: Preparation of a starburst polymer of two full generations from N-t-butoxycarbonyl-6-(4-aminobenzyl)-1,4,8,11-tetraaza-5,7-dioxoundecane

The one and one half generation product (Example S) (3.9 g, 2.5 mmol) was dissolved in 50 ml of methanol, and was added dropwise over 2 hours to a stirred solution of ethylenediamine (600 g, 10 mol) and methanol (50 ml). The solution was stirred at ambient temperatures under an atmosphere of nitrogen for 96 hours. The ethylenediamine/methanol was removed on the rotary evaporator to give a yellow glassy solid (4.4 g contaminated with ethylenediamine). A 10 percent solution of the product was made in methanol, and purified from the ethylene diamine by reverse osmosis (membrane used as a Filmtec FT-30, in an Amicon TC1R thin channel separator) until no ethylenediamine could be detected by gas chromatography (Column, Tenax 60/80. Removal of the solvent gave the product as a yellow glassy solid (3.52 g). The 13C NMR data and HPLC analysis (PLRP-S column, acetonitrile:0.015 M NaOH, 10 to 20 percent gradient in 20 minutes, were consistent with the proposed structure.

Example U: Reaction of the two generation starburst with Bromoacetic Acid to give a methylene carboxylate terminated starburst dendrimer

The second generation product (Example T) (0.22 g, 0.13 mmol) was dissolved in 15 ml of deionized water and the temperature equilibrated at 40.5°C. Bromoacetic acid (0.48 g, 3.5 mmol) and lithium hydroxide (0.13 g, 3.3 mmol) were dissolved in 5 ml of deionized water, and added to the reaction mixture. The reaction pH was carefully maintained at 9, with the use of a pH stat (titrating with 0.1N NaOH), at 40.5°C overnight.

Monitoring by reverse phase HPLC, (Spherisorb ODS-1 column, eluent 0.25 M H_3PO_4 pH 3 [NaOH]; acetonitrile 85:15) confirmed the synthesis of predominantly a single component.

Example V: Preparation of Isothiocyanato functionalized second generation methylene-carboxylate terminated starburst dendrimer

Five ml of a 2.8 mM solution of the second generation methylenecarboxylate terminated starburst dendrimer (Example U) was diluted with 20 ml water and the pH adjusted to 0.5 with concentrated hydrochloric acid. After one hour at room temperature the mixture was analyzed by HPLC to verify the removal of the butoxycarbonyl group and then treated with 50 percent sodium hydroxide to being the pH to 7. A pH stat (titrating with 0.1 N NaOH) was used to maintain the pH at 7 and 225 µl thiophosgene was added. After 15 minutes at room temperature the pH of the mixture was adjusted to 5 with 1N HCl. The mixture washed with chloroform (20 ml x 2) then concentrated on a rotary evaporator at reduced pressure. The residue recovered 0.91 g is a mixture of the isothiocyanate and salts.

Example W: Preparation of second generation starburst polyethyleneimine-methane sulfonamide

To a solution of 125 g N-methanesulfonylaziridine in 50 ml ethanol was added 25.0 g tris(2-aminoethyl)amine. The solution was stirred at room temperature for 4 days. Water was added to the reaction mixture as needed to maintain the homogeneity of the solution. The solvent was removed by distillation *in vacuo* to give the 2nd generation

starburst PEI-methane sulfonamide as a yellow glass (161 g).

Example X: Cleavage of methane sulfonamides to form second generation starburst polyethyleneimine

A solution of 5.0 g of second generation starburst PEI-methane sulfonamide, from Example W in 20 ml of 38 percent HCL was sealed in a glass ampoule. The ampoule was heated at 160°C for 16 hours, then cooled in an ice bath and opened. The solvent was removed by distillation *in vacuo* and the residue dissolved in water. After adjusting the pH of the solution to greater than or equal to 10 with 50 percent NaOH, the solvent was removed by distillation *in vacuo*. Toluene (150 ml) was added to the residue and the mixture heated at reflux under a Dean-Stark trap until no more water could be removed. The solution was filtered to remove salts and the filtrate concentrated *in vacuo* to give 1.9 g second generation starburst PEI as a yellow oil.

Example Y: Preparation of third generation starburst polyethyleneimine-methane sulfonamide

To a solution of 10.1 g second generation starburst PEI, from Example X, in 100 ml ethanol was added 36.6 g N-methanesulfonylaziridine. The solution was stirred at room temperature for 1 week. Water was added as needed to maintain the homogeneity of the solution. The solvent was removed by distillation *in vacuo* to give third generation starburst PEI-methane sulfonamide as a yellow glass (45.3 g).

Example Z: Cleavage of methane sulfonamides to form 3rd gen starburst polyethyleneimine

The methane sulfonamide groups of third generation starburst PEI-methane sulfonamide (5.0 g), from Example Y, were removed by the same procedure as described for the second generation material in Example X to give 2.3 g third generation starburst PEI as a yellow oil.

Example AA: Preparation of a methylenecarboxylate-terminated second generation starburst polyamidoamine (initiated from ammonia)

The second generation starburst polyamidoamine (2.71 g, 2.6 mmol) and bromoacetic acid (4.39 g, 31.6 mmol) were dissolved in 30 ml of deionized water and the pH adjusted to 9.7 with 5N NaOH using a pH stat. The reaction was maintained at this pH for a half hour, and the temperature was slowly raised to 60°C and was maintained at 60°C for three hours at constant pH. The pH was raised to 10.3, and the reaction mixture remained under control of the pH stat at ambient temperatures overnight. The reaction mixture was refluxed for a further four hours prior to work up. Removal of the solvent, and azeotroping the final traces of water with methanol gave the product as a pale yellow powder (8.7 g, contaminated with sodium bromide). The ^{13}C NMR spectrum was consistent with the proposed structure (with some contamination due to a small amount of defected material as a result of some monoalkylation).

Example BB: Preparation of a methylenecarboxylate terminated second generation starburst polyethyleneimine (initiated from ammonia)

The second generation starburst polyethyleneimine (2.73 g, 6.7 mmol), from Example AA, and bromoacetic acid (11.29g, 81 mmol) were dissolved in 30 ml of deionized water. The pH was slowly raised to pH 9.5 maintaining the temperature below 30°C. The temperature was raised slowly to 55°C, and the reaction pH maintained at 9.5 for 6 hours with the aid of a pH stat (titrating with 5N NaOH). The pH was raised to 10.2, and maintained at that pH overnight. Removal of the solvent on the rotary evaporator, and azeotroping the final traces of water using methanol, gave the product as a yellow powder (17.9 g, contaminated with sodium bromide). The ^{13}C NMR spectrum was consistent with the proposed structure (with some contamination due to a small amount of defected material as a result of some monoalkylation).

Example CC: Preparation of a 3.5, 4.5, 5.5 and 6.5 generation starburst PAMAM

To a 10 wt% methanolic solution of 2.46 g 3 generation PAMAM starburst was added 2.32 g of methyl acrylate. This mixture was allowed to sit at room temeprature for 64 hr. After solvent and excess methyl acrylate removal, 4.82 g of product was recovered (105% of theoretical).

Preparation of higher 1/2 generation starburst PAMAM'S:

Generations 4.5, 5.5 and 6.5 were prepared as described above with no significant differences in

reactant concentrations, reactant mole ratios or reaction times.

Example DD: Preparation of a 4, 5 and 6 generation starburst PAMAM:

To 2000 g of predistilled ethylenediamine was added 5.4 g of 4 1/2 generation starburst PAMAM as a 15 wt% solution in methanol. This was allowed to sit at room temperature for 48 hrs. The methanol and most of the excess ethylenediamine were removed by rotary evaporation under water aspirator vacuum at temperature less than 60°C. The total wt of product recovered was 8.07 g. Gas chromatography indicated that the product still contained 34 wt% ethylenediamine at this point. A 5.94 g portion of this product was dissolved in 100 ml methanol and ultrafiltered to remove the residual ethylenediamine. The filtration was run using an Amicon TC1R thin channel recirculating separator equipped with an Amicon YM2 membrane. An in-line pressure relief valve was used to maintain 55 psig (380 kPa) pressure across the membrane. The 100 ml was first concentrated to 15 ml by forcing solvent flow exclusively through the membrane. After this initial concentration, the flow was converted to a constant volume retentate recycle mode for 18 hrs. After this time, 60 ml of methanol was passed over the membrane to recover product still in the module and associated tubing. The product was stripped of solvent and 2.53 g of 5 generation starburst PAMAM was recovered. Analysis by gas chromatography indicated 0.3% residual ethylenediamine remained in the product.

Preparation of generation 4 and 6 proceeded as above with the only difference being the weight ratio of ethylenediamine to starting material. To prepare 4th generation this ratio was 200:1 and for 6th generation this ratio was 730:1.

<u>Example 1:</u> Preparation of a product containing more than one rhodium atom per starburst polymer.

2.5 Gen PAMAM (ester terminated, initiated off NH_3) (0.18 g, 0.087 mmole) and $RhCl_3{\bullet}3H_2O$ (0.09 g, 0.3 mmole) were mixed in dimethylformamide (DMF) (15 ml) and heated for 4 hours at 70°C. The solution turned crimson and almost all of the rhodium was taken up. The unreacted rhodium was removed by filtration and the solvent removed on the rotary evaporator. The oil formed was chloroform soluble. This was washed with water and dried ($MgSO_4$) before removal of solvent to yield a red oil (0.18 g). The NMR spectrum was recorded in $CDCl_3$ only minor differences were noted between the chelated and unchelated starburst. Dilution of some of this $CDCl_3$ solution with ethanol followed by $NaBH_4$ addition resulted in rhodium precipitation. $RhCl_3{\bullet}3H_2O$ is insoluble in chloroform and in chloroform starburst solution thus confirming chelation.

<u>Example 2:</u> Preparation of a product containing Pcl chelated with a starburst polymer

3.5 Generation PAMAM (ester terminated, initiated off NH_3) (1.1 g, 0.24 mmole) was dissolved with stirring into acetonitrile (50 ml). Palladium chloride (0.24 g, 1.4 mmole) was added and the solution was heated at 70-75°C (water bath) overnight. All the $PdCl_2$ was taken up into the starburst. Removal of the

solvent and recording the NMR in $CDCl_3$ confirmed that chelation had occurred. Dilution of the $CDCl_3$ solution with ethanol and addition of $NaBH_4$ resulted in precipitation of the palladium. The chelated product (1.23 g) was isolated as a brown oil.

Example 3: Preparation of a product containing fluoroscein with a starburst polymer

A sample of 5-carboxyfluorescein (0.996 g) and starburst polyethyleneimine (Gen=2.0; amine terminated, initiated off NH_3) (0.202 g) were mixed in 10 ml of methylene chloride and 5 ml of methanol and allowed to reflux for 10 minutes. Upon filtering, an insoluble red powder (0.37 g) was obtained (mostly unreacted 5-carboxy fluorescein). From the filterate was isolated 0.4 g of a brilliant-red solid which exhibited a softening point of 98-103°C and foamed to a brilliant red melt at 175-180°C; NMR spectra (D_2O) of this product were consistent with dendrimer having fluoroscein bound to the surface.

Example 4: Preparation of a product containing fluoroscein with a starburst polymer

In a procedure similar to that described in Example 3, starburst polyethyleneimine (Gen=2.0; amine terminated, initiated off NH_3) was reacted with fluorescein isothiocyanate to give a brilliant-red iridescent solid which was suitable for use as a fluorescent labelling reagent.

Example 5: Hydrolysis of 4.5 generation dendrimers and preparation of calcium salt.

4.5 Generation PAMAM (ester terminated, initiated off NH_3) (2.11 g, 10.92 meq) was dissolved in 25 ml of methanol and to it was added 10% NaOH (4.37 ml, 10.92 meq) (pH = 11.5-12). After 24 hours at room temperature, the pH was about 9.5. After an additional 20 hours, the solution was rotovaped, 50 ml of toluene added, and rotovaped again.

The resulting oil was dissolved in 25 ml of methanol and precipitated as a white gum upon addition of 75 ml of diethyl ether. The liquid was decanted off, and the gum was rotovaped extensively to give a very fine off-white powder which upon further drying gives 2.16 g of product (98% yield). No ester groups were found upon NMR and infrared analysis.

The sodium salt of 4.5 Generation PAMAM (ester terminated, initiated from NH_3) was exchanged for the calcium salt via dialysis. The sodium salt (1.03 g) was dissolved in 100 ml of water and passed through hollow fiber dialysis tubing (cut off = 5000) at 3 ml/minute. The exterior of the tubing was bathed in 5% $CaCl_2$ solution. This procedure was then repeated.

The resulting solution was again dialyzed, this time against water, then repeated two additional times.

Evaporation provided 0.6 g of wet solid, which was taken up in methanol (not totally soluble) and is dried to give 0.45 g of off-white crystals.

$C_{369}H_{592}O_{141}N_{91}Ca_{24}$ Calc. - 10.10% Ca^{++}

M Wt. = 9526.3 Calc. = C-4432.1, H-601.8, O-2255.9, N-1274.6, Ca-961.9)

Theo: C-46.5, H-6.32, N-13.38, Ca-10.10
Found: C-47.34, H-7.00, N-13.55, Ca-8.83

Example 6: Preparation of dendrimers with terminal carboxylate groups.

Half-generation starburst polyamidoamines were hydrolyzed to convert their terminal methyl ester groups to carboxylates. This generated spheroidal molecules with negative charges dispersed on the periphery. The dendrimers hydrolyzed ranged from 0.5 generation (three carboxylates) to 6.5 generation (192 carboxylates).

The products could be generated as Na^+, K^+, Cs^+ or Rb^+ salts.

Example 7: Encapsulation of R(+) - Limonene in Polyamidoamine Starburst Dendrimers

A 5-50 weight percent solids solution in methanol of starburst - PAMAM dendrimer (M.W. about 175,000; generation = 9.0) was added dropwise to (R(+) limonene in methanol until saturated. The solution was stirred at room temperature (about 25°C) for several hours and then devolatized on a Büchi rotovap at room temperature to give a solid product. Warming at temperatures greater than 80°C gave solvent insoluble products which retained substantial amounts of (R+)-limonene in an encapsulated form. These products are excellent prototypes for slow release of (R+)-limonene as a fragrance and deodorizer product.

Example 8: Encapsulation of Heavy Metal Salts in Polyamindoamine starburst Dendrimers

A 5-50 weight percent solids solution in water of starburst PAMAM dendrimer (M.W. about 350,000; generation = 10.0) was stirred as a saturated solution of lead acetate [$Pb(C_2H_3O_2)_2$] is added dropwise. The solution was stirred at room temperature (about 25°C) for several hours and then devolatilized or a Büchi rotorap to give solid products. Scanning transmission electromicrograph of these products showed that these heavy metal salts are encapsulated in the interior of the dendrimers. These films containing heaving metal salts are useful as shields for absorbing electromagnetic radiation.

Example 9: Encapsulation of Fluorescein (water soluble) Dye in Polyamidoamine Starburst Dendrimers

A 5-50 weight percent solids solution (H_2O/CH_3OH) of starburst-PAMAM dendrimer (M.W. about 175,000; generation =9.0) was stirred as fluorescein, disodium salt (Acid Yellow 73, Cl. 45350; Uranine; available from Aldrich Chemical Co. (Milwaukee, WI) is added until saturated. The solution was stirred at room temperature (about 25°C) for several hours and then devolatilized at room temperature to give a colored solid product. These dye encapsulated dendrimers are excellent reference probes for calibrating ultrafiltration membranes.

Example 10: Preparation of dendrimers with terminal fluorescent groups

A. Reaction of Amine Terminated Dendrimer with N-Dansyl Aziridine

A sample (1.5 g, 1.6×10^{-3} mole) of starburst polyethyleneimine (LPEI), G = 3.0, terminal groups (Z) = 12, M.W. = 920) was dissolved in 20 ml of methanol. The solution was stirred and 0.884 g (3.84×10^{-2} mole) of a solution of N-dansyl aziridine (ICN Biomedicals, Costa Mesa, CA) was added dropwise over a period of 20 minutes. The reaction mixture was allowed to stir at room temperature overnight. Removal of solvent under vacuum gave a solid product. NMR and infrared analysis indicated that the product was covalently bonded dansyl groups in the surface of the dendrimer.

B. Reaction of Amine Terminated Dendrimers with Dansyl Chloride

A solution of starburst polyamidoamine (1.0 g, 1.9×10^{-4} mole) (initiated from ammonia, G = 4.0, terminal groups (Z) = 24, M.W. = 5,147) in 30 ml of water was stirred in a 3-neck flask with 80 ml of toluene while a solution of dansyl chloride (1.23 g, 4.5×10^{-3} mole) (5-dimethyl-amino-1-naphthalenesulfonyl chloride, from Aldrich Chemical Co., Milwaukee WI) in 40 ml of toluene was added dropwise while cooling with ice. Concurrently, a solution of 10% NaOH (13.3 mole, 10% excess) was added to the reaction mixture to give an oily ball. The product was washed with water, dissolved in methanol, and precipitated with diethyl ether to give a solid product. NMR and infrared analysis was consistent with covalently bonded dansyl groups in the dendrimer surface.

Example 11: Demonstration of multiple chelation of iron by a sodium propionate terminated sixth generation starburst polyamidoamine.

The sodium propionate terminated sixth generation polyamidoamine (initiated from ammonia) (97.1 mg, 2.45 mol.) was dissolved in 1.5 ml of deionized water. Addition of 0.5 ml of 0.5N HCl reduced the pH to 6.3. Ferric chloride was added (0.5 ml of 0.1.2M solution, 0.051 mmol) producing a light brown gelatinous precipitate. On heating at 60°C for 0.5 hours, the gelatinous precipitate became soluble, resulting in a homogeneous orange solution. The solution was filtered through Biogel P2 acrylamide gel (10 g, twice) isolating the orange band (free of halide contamination). Removal of the solvent in vacuo gave the product as an orange film (30 mg). Analysis was consistent with chelation of approximately 20 moles of ferric ions per mole of starburst dendrimer.

Table III

Found	Theoretical		
	$Na_4Fe_{20}H_{128}SB$	$Na_5Fe_{20}H_{127}SB$	$Na_6Fe_{20}H_{126}SB$
Na 0.39,0.24 (0.31 0.1%)	0.25	0.31	0.38
Fe 3.14,3.11 (3.12 0.02%)	3.05	3.05	3.04
C 47.11	49.87	49.84	49.81
H 7.33	7.31	7.30	7.29
N 14.81	14.49	14.48	14.47
O ----	25.03	25.02	25.01
Mwt.	36632.23	36654.21	36375.18

SB = $C_{1521}H_{2467}N_{379}O_{573}$

These results confirm chelation of 20±2 moles of ferric ions per mole of starburst dendrimer.

1. A starburst conjugate which comprises at least one starburst polymer associated with at least one unit of at least one carried material.

2. The conjugate of Claim 1 wherein the starburst polymer is a starburst dendrimer.

3. The conjugate of Claim 1 or 2 wherein at least one of the carried materials is a signal generator, signal reflector, or signal absorber.

4. The conjugate of Claim 2 wherein there are at least two different carried materials, at least one of which is a target director.

5. The conjugate of Claim 1 wherein the dendrimer contains discontinuities.

6. A starburst conjugate of Claim 1 of the formula:

$$(P)_x * (M)_y$$

wherein each P represents a dendrimer;

x represents an integer of 1 or greater;

each M represents a unit of a carried material, said carried material can be the same carried material or a different carried material;

y represents an integer of 1 or greater; and

* indicates that the carried material is associated with the dendrimer.

7. The conjugate of Claim 6 wherein M is signal reflector, or signal absorber.

8. The conjugate of Claim 6 wherein x=1 and y=2 or more.

9. The conjugate of Claim 7 wherein y=2 or more.

10. The starburst conjugate of Claim 6 wherein the molar ratio of any ionic M to P is 0.1-1,000:1.

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11. The starburst conjugate of Claim 6 wherein the weight ratio of any pesticide or toxin M to P is 0.1-5:1.

12. A starburst conjugate composition which comprises one or more starburst conjugates of any one of Claims 1 to 11 and at least one suitable diluent or carrier.

13. A starburst conjugate of any one of Claims 1 to 12 for use as a carrier for a dye, fragrance,

fluorescing entity, paramagnetic entity, pheromone or election beam opacifier.

14. A process for preparing

$$(P)_x \quad * \quad (M)_y \qquad\qquad (I)$$

wherein each P represents a dendrimer; x represent an integer of 1 or greater; each M represents a unit of a carried material, said carried material can be the same carried material or a different carried materal; y represents an integer of 1 or greater; and * indicates that the carried material is associated with the dendrimer, which comprises reacting P with M, usually in a suitable solvent, at a temperature which facilitates the association of the carried agricultural material (M) with the starburst dendrimer (P).

15. The process of Claim 14 wherein the temperature is from room temperature to reflux.

16. The process of Claim 14 wherein the suitable solvent is water, methanol, ethanol, chloroform, acetonitrile, toluene, dimethylsulfoxide or dimethylformamide.

AMENDED CLAIMS

[received by the International Bureau on 12 January 1988 (12.01.88) new claims 17 - 23 added ; other claims unchanged (4 pages)]

17. The conjugate of Claim 2 wherein the starburst dendrimer is of the formula

$$(\text{Core}) \left\langle \left((\text{Repeat Unit})_{\frac{N_r^{G}-1}{N_r-1}} \; \text{Terminal Moiety}_{N_r^{G}} \right\rangle \right)_{N_c}$$

wherein: the core is

# of terminal groups per dendritic branch =

$$\frac{N_r^{G}}{2};$$

G is the number of generations; N_r is the repeating unit multiplicity which is at least 2; N_c is the valency of the core compound; the terminal moiety is determined by the following:

# of terminal moieties per dendrimer =

$$\frac{N_c N_r^{G}}{2}$$

wherein N_r, G and N_c are as defined above; and the Repeat Unit has a valency or functionality of $N_r + 1$ wherein N_r is as defined above.

18. The conjugate of Claim 2 wherein the starburst dendrimer is of the formula

$$\left(\textcircled{\small I}\,(Z^c)_{N_c}\right)\left\{\left(X^iY^i(Z^i)_{N^i}\right)^{i-1}_{\substack{N_c\Pi N^n \\ n\text{ is }1}}\right\}\left(X^tY^t(Z^t)_{N^t}\right)^{t-1}_{\substack{N_c\Pi N^n \\ n\text{ is }1}}$$

wherein i is 1 to t-1; the core compound is represented by the formula

$$\textcircled{\small I}\,(Z^c)_{N_c}$$

where

$$\textcircled{\small I}$$

represents the core, Z^c represents the functional groups bonded to

$$\textcircled{\small I}$$

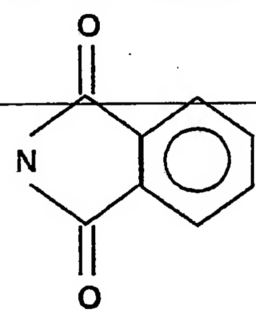
and N_c represents the core valency; the repeat unit is represented by the formula $X^iY^i(Z^i)_{N^i}$ wherein "i" is defined as above; the final or terminal units are represented by $X^tY^t(Z^t)_{N^t}$ wherein t represents terminal

generation and X^t, Y^t, Z^t and N^t may be the same as or different from X^i, Y^i, Z^i and N^i except that there is no succeeding generation connected to the Z^t groups and N^t may be less than two; the n function is the product of all the values between its defined limits, such as

$$\prod_{n=1}^{i-1} N^n = (N^1)(N^2)(N^3)\ldots(N^{i-2})(N^{i-1})$$

which is the number of repeat units, $X^iY^i(Z^i)_{N^i}$, comprising the ith generation of one dendritic branch and when i is 1, then $\prod_{n=1}^{o} = 1$.

19. A process for preparing a starburst conjugate as defined in Claim 1 which comprises the reaction of P, having reactive moieties, with an aniline moiety, which may have the NH_2 group protected by an N-phthalimide of the formula



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20. A process for preparing a starburst conjugate as defined in Claim 1 which comprises the reaction of P, having reactive moieties, which may have the NH_2 group protected by any protecting group used for amines which is inert under the conditions used for starburst synthesis.

21. A process for preparing a starburst polyethyleneimine which comprises reacting a starburst polyethyleneiminemethane sulfonamide with hydrochloric acid.

22. A process for purifying a starburst dendrimer having a solvent present which comprises removing the solvent by ultrafiltration using a membrane.

23. The process of Claim 22 wherein the solvent is ethylenediamine.

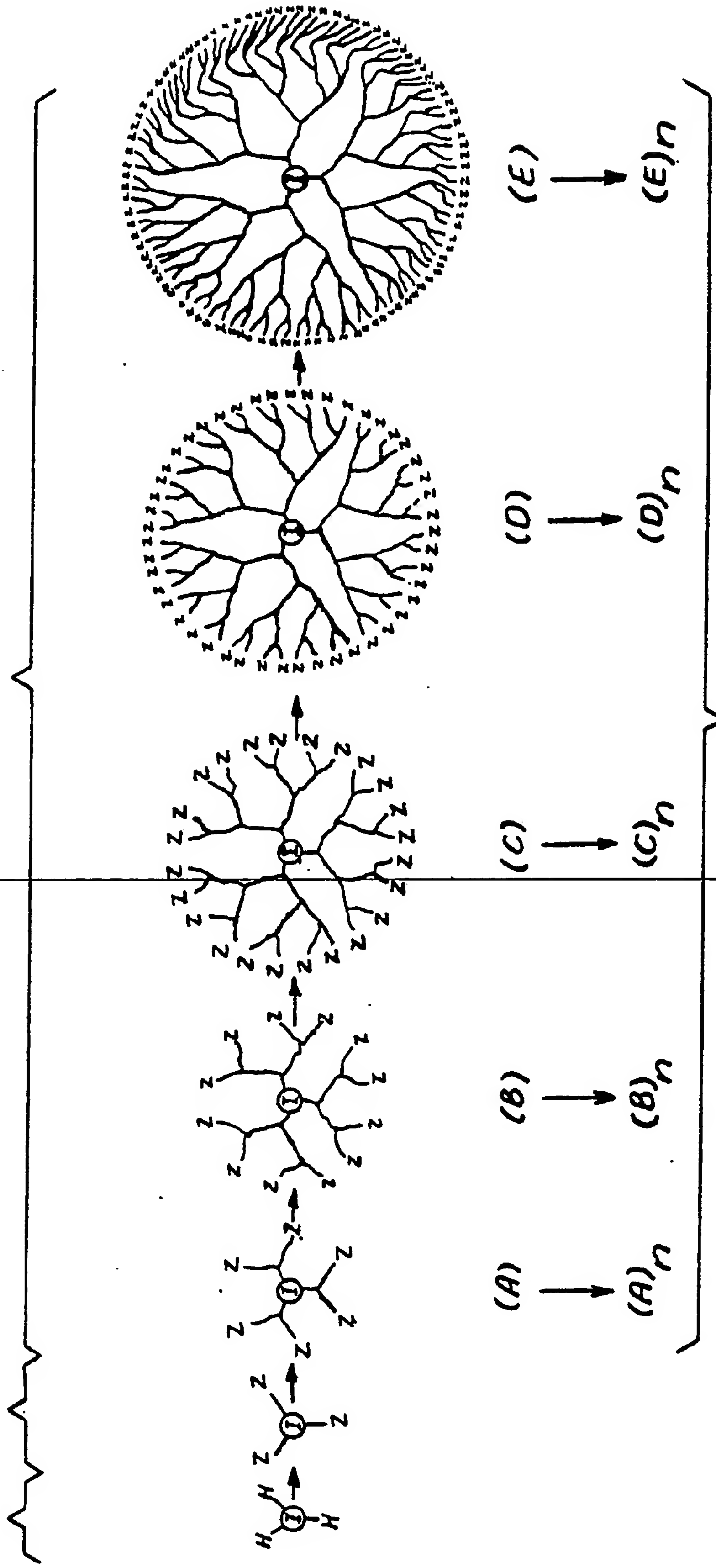

Fig. 1

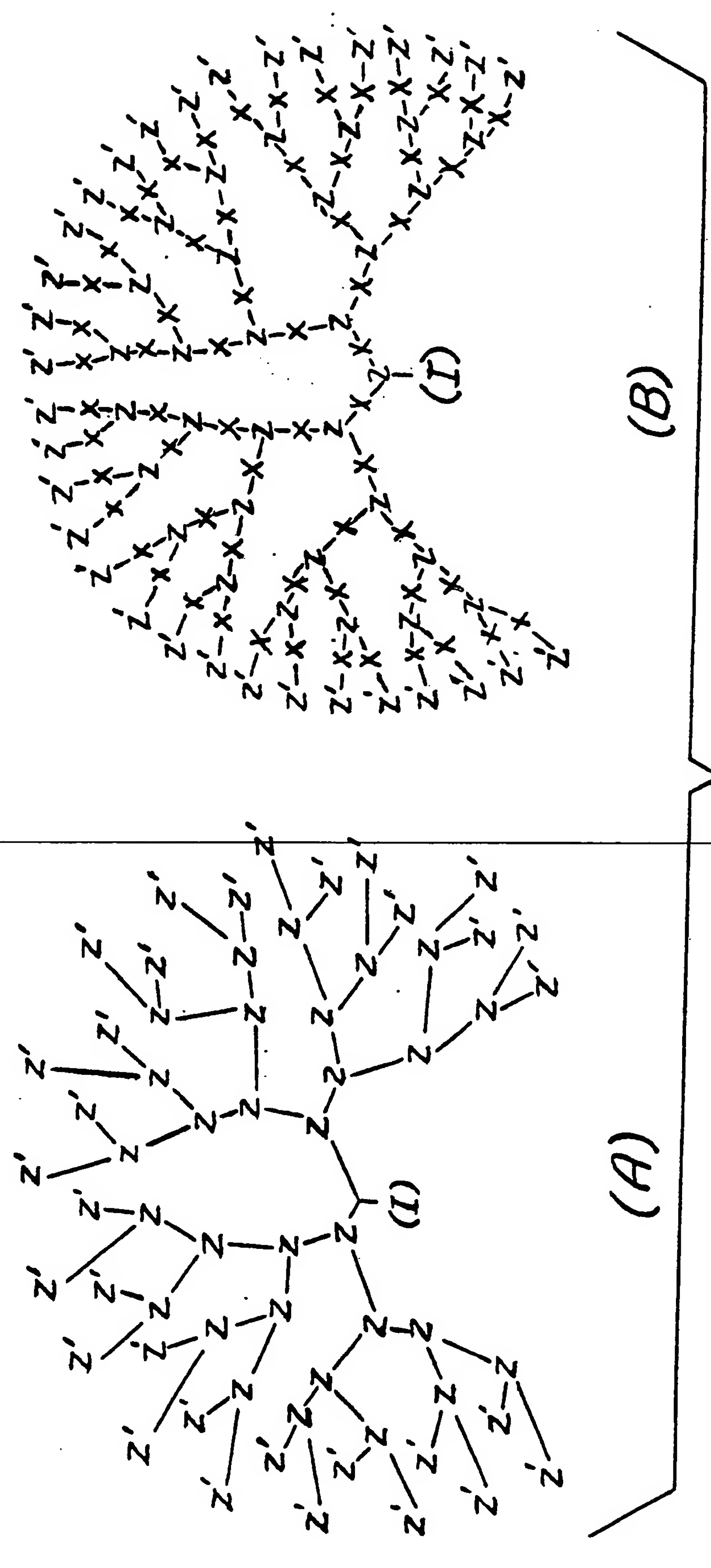

Fig. 2

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 87/02076

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

INT.CL. 4 A61K 49/02
US. CL. 424/1.1

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	424/1.1,9 525/410,416,418,451 528/310,332,350,363,397

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X Y	US, A, 4,558,120 PUBLISHED 10 DECEMBER 1985 TOMALIA ET AL (Col. 12, lines 16-41)	1,2,5,6 8,10-16
P,A	US, A, 4,606,907 PUBLISHED 19 AUGUST 1986 SIMON ET AL	1-16
T	US, A, 4,694,064 PUBLISHED 15 SEPTEMBER 1987 TOMALIA ET AL	1-16

* Special categories of cited documents: [15]
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
29 OCTOBER 1987	02 DEC 1987
International Searching Authority [1]	**Signature of Authorized Officer** [20]
ISA/US	John S Maples John Maples